A Study on a Novel Analytical Method Development and Validation of Pharmaceutical Dosage Forms by using RP-HPLC Method

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Abstract

A number of pharmaceutical drugs are methods are considered to be rapid, accurate, precise, specific, and offers the ease of automation being introduced into the arcade every year in a cumulative phase. Some of these drugs either may be a new entity or a structural modification of existing molecular formulation in multi combination of two or more drug substances. Very often these new drugs or drug combinations are not included in the pharmacopoeias from the date of introduction into the market. This is happening due to the post phase clinical studies conducted to understand the toxicity and patient resistance which results in the introduction of a better drug by competitors. Under these conditions the standard and analytical procedures may not be presented in the official pharmacopoeias. Hence new method development for simultaneous determination and validation for such drugs has become necessary.

Keywords: *pharmaceutical drugs, HPLC Apparatus, Methanol.*

I. INTRODUCTION

No official method available in pharmacopoeia for the drug or drug combinations. Available methods in the literature are not suitable for the analysis of dosage forms. The procedures of analyzing drug substances are not suitable to carry out analysis of dosage forms due to the interference of excipients present in formulation. Non availability of a method for the quantification of the drug in biological fluids. Analytical methods always being specific to each dosage form, in many cases the available methods of combination with other drug substances are not suitable.

II. HPLC METHOD OF ANALYSIS

Analytical methods available in official pharmacopoeias for the analysis of drug substances either individually or combination with other drug are mostly by HPLC analysis.

This is because methods using HPLC have several advantages over the other methods used for analysis, few of them are

1. Most assay methods can be evaluated within 20 minutes.
2. Sensitivity is high.
3. Required resolution can be achieved within the short run due to wide range of stationary phase.
4. Columns are used for multiple analyses.
5. Handling and maintenance is easy.
6. Accuracy is very high.
7. Automation instruments make the quantification easy with less labor.
8. Suitable for continuous analysis on a large scale (can support continuous production)

Different modes of chromatography like Normal phase (NP), reversed phase (RP), reverse phase with ion pair, chiral, affinity and size exclusion chromatography are very commonly used for the separation of drugs in different dosage forms.

The chromatographic separation is achieved by normal phase or reverse phase chromatographic elution techniques. In the normal phase chromatographic method, the stationary phase is polar and mobile phase is non-polar, this technique is mostly used for the determination of non-polar compounds. Polar compounds are retained in the stationary phase due to the high affinity towards the stationary phase. Hence the normal phase mode of separation is not generally used for the analysis of pharmaceutical drug products due to the polar nature of the pharmaceutical products.

Reversed phase chromatography is most widely used technique for the separation, of drugs and for the

estimation of drug contents in the fields like biomedical sciences, biological industry, chemical industry, food and pharmaceutical industries.

In the Reverse phase method, the stationary phase is non-polar and the mobile phase is a polar. This non-polar stationary phase is a hydrophobic packing with octayldecyl functional group bonded to silica gel. An aqueous phase allows the use of subordinate solute element equipoise (such as ionization rheostat, ion destruction, ion combination and complexation) to control retention and discrimination. As most of the pharmaceutical drug products are polar in nature, which elute faster and have a low affinity towards the non- polar stationary phase, they are detected and separated easily. These columns with non-polar stationary phases are in different forms based on the number of carbon chains C_4, C_8, and C_{18} etc., Octadecyl silane (ODS).

A. Quantitative Analysis by HPLC technique

Four methods are generally used for quantitative analysis. They are

1. Normalized peak area

After integrating all significant peaks in chromatogram, total peak area may be calculated. Area (%) of any individual peak is called standardized peak area. This procedure is extensively used predominantly in initial method development.

% A = Area of Peak A *100 Total area of peaks (A+B+C+D)

2. External standard method

The external standard method involves the use of a single standard for comparison or one can also use the slope of the calibration curve based on standard that contains known concentrations of the compound of interest. The peak area or the height of the sample and the standard used are compared directly.

3. Internal standard method

A widely used technique of quantitation involves the addition of an internal standard to reimburse for the various analytical errors. In this methodology, a known compound of fixed concentration is added to the known amount of sample to given separation peaks in the chromatograms to compensate for the losses of the compounds of interest during sample pre- treatment steps. Any loss of the component of interest will be accompanied by the loss of an equivalent fraction of internal standard. The accuracy of this approach is obviously dependent on the structural equivalence of the compounds of interest and the internal standards. The internal standards should be added to the sample prior to sample preparation procedure and homogenized with it. Response factor (RF) is the ratio of peak area of sample component (A_X) and the internal standard (A_{ISTD}) is used for the quantification of sample in place of area. In general, area from sample chromatogram and standard chromatogram are used to calculate unknown sample quantification. Where the internal standard is added, the response factor of sample chromatogram and response factor of standard chromatogram are used for the sample quantification.

$RF = A_X / A_{ISTD}$

Where

RF = Response factor

A_X = Area of the peak of interest A_{ISTD} = Area of internal standard

When more than one compound is to be quantified from one test sample, response factor for the each component should be determined by using the area of that compound against the area internal standard in calculating quantity of the compounds.

4. Standard addition method

In this method of standard addition, a known amount of the standard is added to the sample solution to be determined. This has been carried out in case where compound of interest is insufficient for detection. In this case, the amount added is estimated first and later the amount after addition together with the sample is estimated.

B. Design and development of HPLC method

Analytical test methods are the important areas in product development. These methods provide reliable and accurate data support to the formulation, characterization, packaging, process development, pharmacokinetics, bio equivalency, release and regulatory filing. The time and effort spent in developing a good test method will help users such as laboratory technicians and scientists in the QC labs of drug manufacture. Hence it is important to understand the objectives and requirements of the test method before the initiation of development of any analytical test method.

A suitable analytical method for the estimation of drugs can be developed by knowing the nature of the sample like polarity, structure, molecular weight, ionic character, solubility parameters etc.

Method Development is a very challenging task. To achieve the desired task every researcher is

confronted with typical questions like, where to start? What type of column is worth trying with kind of mobile phase?

In general most of the pharmaceutical products consist of organic drug molecules which are mostly polar or mid polar in nature, hence reverse phase chromatography is appropriate for their quantification.

In most cases, mobile phase composition is fixed initially. The target run time of the chromatography can be achieved by adjusting the organic composition of mobile phase. If the retention time of chromatogram is too short, organic phase concentration of the mobile phase can be reduced up to 5% to achieve the target run time. If the retention time is too long, organic phase concentration of mobile phase can be increased.

Elution pattern of compounds can be altered by changing the polarity of the mobile phase. Ionic samples (acidic or basic) can be separated by keeping the sample un- dissociated. Dissociation of ionic sample will be suppressed by the proper selection of pH of diluent and mobile phase. The pH of the mobile phase has to be selected in such a way that the compound is not ionized.

To obtain good analytical results in reverse phase chromatography by UV detection, wavelength selection is one of the key parameters. This requires knowledge of UV spectra of each individual components present in the sample. Solubility and UV absorption spectra of test solution help to fix the wavelength for the method.

The molar absorbance at the detection wavelength is also an important parameter. This provides fair idea about required concentration for detection. If the sample quantity is not enough for detection, peaks are not detected in the chromatogram.

It is not mandatory to detect compounds at their wavelength where it shows maximum absorbance. It is based on the requirements in terms of concentration of compounds of interest and their responses. When acceptable peaks are detected in the chromatogram, further method development trails help to investigate for the better peak shapes. If there is no UV absorbance for any of the compounds of interest, they can be converted into a UV active compound by derivatization.

If the peak shows tailing or fronting, it is obvious that the mobile phase is not well- matched with the solute. In most cases, the pH is not properly selected, hence partial dissociation or protonation takes place. Where there is no possibility to achieve proper peak shape even at low or high pH for ionic compounds, ion-pair chromatography is suggested. For acidic compound, cationic ion pair molecules at higher pH and for basic compounds, anionic ion-pair molecules at lower pH can be used. For amphoteric solutes or a mixture of acidic and basic compounds, ion-pair chromatography is the most preferred choice.

The bad peak shape may be due to the low solubility of the sample. It is commonly advised to use mobile phase for the preparation of the sample and their dilutions. This avoids the precipitation of compounds in the injector or column.

Optimization of chromatographic conditions shall continue after acquiring a sensible chromatogram. An augmented chromatogram is the one in which all the peaks are symmetrical and are well detached.

III. HPLC METHOD VALIDATION AND GUIDELINES

The U.S FDA has provided manufacturing directions for Analytical Measures and Method Authentication. The ICH guidance provides clear text on the validation of analytical procedures.

The USP has precise strategies available for method validation for compound assessment. USP describes eight steps for endorsement.

Accuracy, Precision, Specificity, Limit of Detection, Limit of quantification, Linearity and range, Ruggedness, Robustness The Analytical method validations criteria were defined based on the purpose of their use. Details of categories and requirements are presented below.

A. Precision

The precision of an analytical method is the degree of agreement among individual test results obtained when the method is applied to multiple sampling of a homogenous sample. The Precision is calculated as coefficient of variation (C.V) i.e., relative standard deviation (RSD). The measured RSD can be subdivided into three categories; repeatability (Intra-day precision), intermediate precision (inter-day precision) and reproducibility (between laboratories).

Repeatability is the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time. Intermediate precision refers to the results within lab variations due to random changes such as difference in analysts, equipment, experimental period and so forth.

Reproducibility is the closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipment. Documentation in support of precision and intermediate studies should include the standard deviation, relative standard deviation, coefficient of variation, and confidence interval.

B. Specificity

The specificity is defined as the ability to measure accurately the concentration of an analyte in the presence of all other sample materials. If specificity is not assured; method precision, accuracy and linearity are all seriously compromised. Method specificity should be reassessed continually during validation and subsequent use of the method. Specificity determination can be achieved in two ways.

C. Accuracy

Accuracy is the measure of the closeness of test results obtained by a method to the true value. It indicates the deviation between the mean value found and the true value. Accuracy is represented and determined by recovery studies. Accuracy determination for an HPLC Method should be carried out with a minimum of nine measurements using at least three concentration levels. This approach minimizes any variability or bias in the sample preparation technique and analysis for one sample at only one concentration.

D. Limit of Quantification

The limit of quantification can be defined as the smallest concentration of analyte which gives a response that can be determined with acceptable precision and accuracy. There are different types of acceptable approaches according to ICH to identify the limit of quantification.

E. Based on Visual Evaluation

This method can be used for both instrumental and non-instrumental methods. Generally determined by analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

F. Based on Signal-to-Noise Approach

The signal to noise ratio approach can be applied to analytical procedures that exhibit base line noise. The signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

The quantification limit (QL) may be expressed as

$QL = 0\sigma/S$

Where σ = the standard deviation of the response

S= Slope of the calibration curve

G. Limit of Detection

Limit of detection is the smallest level of analyte that gives a measurable response, but not necessarily quantitated under the stated experimental conditions. It is important for impurity tests and the assays of dosage containing low drug levels and placebos.

H. Based on Signal-to-Noise Approach

The signal to noise ratio approach can be applied to analytical procedures that exhibit base line noise. The signal to noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected. A typical signal-to-noise ratio is 3 or 2:1.

The detection limit (DL) may be expressed as $DL = 3.3\sigma/S$, Where

σ = Standard deviation of the response S = Slope of the calibration curve

The signal can be increased by selecting the optimum monitoring wavelength, increasing injection volume, increasing the peak sharpness with high efficiency columns and by optimizing the mobile phase. The noise can be reduced by using high sensitive detectors with low noise and drift characteristics, mobile phase with low absorbance and pumps with low pulsation and slower detector response time.

I. Linearity and Range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of the analyte in the sample. A linear relationship should be evaluated across the range of the analytical procedure.

J. Robustness

The robustness of an analytical procedure has been defined as a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters and provides an indication of its reliability during normal usage.

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